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Supporting information for article:

**Instrumentation and Experimental Procedures for Robust
Collection of X-ray Diffraction Data from Protein Crystals across
Physiological Temperatures**

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Temperature (K)	Proteinase K							
	293	293	333	333	333	343	343	343
Wavelength	0.95369	0.95369	0.95369	0.95369	0.95369	1.03316	1.03316	1.03316
Resolution range (Å)	34.94-1.55 (1.58-1.55)	34.99-1.08 (1.10-1.08)	35.28-1.50 (1.53-1.50)	35.11-1.40 (1.43-1.40)	35.17-1.40 (1.42-1.40)	35.53-1.45 (1.47-1.45)	35.29-1.40 (1.42-1.40)	35.34-1.45 (1.47-1.45)
Dose (kGy)	5	10	16	12	9	23	13	14
Collection time (s)	20	20	10	10	10	10	5	5
Dose rate (kGy/s)*	0.25	0.5	1.6	1.2	0.9	2.3	2.6	2.8
Space group	P4 ₃ 2 ₁ 2	P4 ₃ 2 ₁ 2	P4 ₃ 2 ₁ 2	P4 ₃ 2 ₁ 2	P4 ₃ 2 ₁ 2	P4 ₃ 2 ₁ 2	P4 ₃ 2 ₁ 2	P4 ₃ 2 ₁ 2
Unit cell dimensions (Å and °)	67.74 67.74 102.13 90.00 90.00 90.00	67.90 67.90 102.18 90.00 90.00 90.00	68.18 68.18 103.55 90.00 90.00 90.00	67.89 67.89 102.93 90.00 90.00 90.00	68.02 68.02 103.08 90.00 90.00 90.00	68.49 68.49 104.57 90.00 90.00 90.00	68.23 68.23 103.54 90.00 90.00 90.00	68.26 68.26 103.75 90.00 90.00 90.00
Total reflections	258136 (10602)	731044 (31801)	287239 (12547)	348701 (17185)	335008 14595	313164 (13391)	357529 (17185)	314183 (13858)
Multiplicity	7.3 (6.4)	7.1 (6.3)	7.3 (6.5)	7.2 (7.0)	7.1 (6.1)	7.0 (6.3)	7.4 (7.2)	7.1 (6.5)
Mosaicity (°)	0.33	0.07	0.41	0.35	0.48	0.53	0.29	0.47
Completeness (%)	99.9 (97.8)	100 (99.9)	99.4 (98.6)	99.9 (98.2)	98.3 (97.8)	99.7 (97.2)	99.4 (96.8)	99.7 (98.2)
Mean I/sigma(I)	8.6 (0.7)	8.1 (0.7)	7.5 (0.6)	6.0 (0.5)	5.8 (0.7)	5.7 (0.7)	9.3 (0.7)	6.0 (0.6)
Wilson B-factor (Å ²)	25.8	13.7	25.9	25.1	23.6	26.3	24.2	25.0
R-merge	0.143 (2.300)	0.115 (2.568)	0.159 (3.482)	0.201 (5.457)	0.144 (2.347)	0.139 (2.806)	0.117 (3.415)	0.147 (3.334)
R-pim	0.056 (0.970)	0.046 (1.085)	0.062 (1.410)	0.078 (2.104)	0.054 (0.984)	0.053 (1.184)	0.044 (1.286)	0.060 (1.362)
CC _{1/2}	99.9 (32.4)	99.9 (33.5)	99.9 (32.9)	99.9 (33.3)	99.9 (32.5)	99.9 (34.0)	99.9 (30.5)	99.9 (34.1)
Isa	37.9	21.4	32.6	16.4	17.0	16.1	31.2	25.2

Temperature (K)	Proteinase K					
	353	353	353	363	363	363
Wavelength (Å)	1.03316	1.03316	1.03316	1.03316	1.12709	1.12709
Resolution range (Å)	35.51-1.65 (1.68-1.65)	35.54-1.50 (1.53-1.50)	35.38-1.65 (1.68-1.65)	35.61-1.65 (1.68-1.65)	35.46-1.68 (1.71-1.68)	35.64-1.66 (1.69-1.66)
Dose (kGy)	14	15	15	29	32	32
Collection time (s)	5	5	5	10	10	10
Dose rate (kGy/s)*	2.8	3.0	3.0	2.9	3.2	3.2
Space group	P4 ₃ 2 ₁ 2	P4 ₃ 2 ₁ 2	P4 ₃ 2 ₁ 2	P4 ₃ 2 ₁ 2	P4 ₃ 2 ₁ 2	P4 ₃ 2 ₁ 2
Unit cell dimensions (Å and °)	68.32 68.32 104.76 90.00 90.00 90.00	68.48 68.48 104.65 90.00 90.00 90.00	68.23 68.23 104.11 90.00 90.00 90.00	68.50 68.50 105.04 90.00 90.00 90.00	68.23 68.23 104.58 90.00 90.00 90.00	68.47 68.47 105.32 90.00 90.00 90.00
Total reflections	218638 (9069)	291337 (13023)	220099 (9752)	216616 (8631)	206719 (9444)	216105 (9487)
Multiplicity	7.1 (6.2)	7.2 (6.7)	7.5 (6.6)	7.0 (5.9)	7.2 (6.6)	7.1 (6.7)
Mosaicity (°)	0.51	0.51	0.42	0.56	0.50	0.52
Completeness (%)	99.9 (98.2)	99.6 (98.1)	97.7 (97.2)	99.8 (97.3)	99.8 (97.5)	99.8 (96.7)
Mean I/sigma(I)	6.1 (0.6)	6.0 (0.5)	5.8 (0.6)	5.5 (0.4)	6.5 (0.4)	6.7 (0.6)
Wilson B-factor (Å ²)	31.3	27.3	31.1	33.8	35.0	34.1
R-merge	0.152 (3.003)	0.161 (4.339)	0.174 (2.298)	0.168 (4.714)	0.182 (5.599)	0.151 (4.087)
R-pim	0.060 (1.271)	0.063 (1.707)	0.065 (0.928)	0.067 (2.058)	0.070 (2.162)	0.060 (1.683)
CC _{1/2}	99.8 (30.1)	99.9 (32.0)	99.9 (36.0)	99.8 (31.5)	99.8 (33.1)	99.9 (36.8)
Isa	23.1	27.2	19.6	15.7	18.7	20.4

Table S1. Diffraction statistics. Proteinase K crystals diffraction statistics are reported for datasets of 100° total rotations, as these were sufficiently complete. Values in parenthesis are for the highest resolution shells. Unit cell parameters were obtained using images from the entire 100°. All statistics were obtained from Aimless (Evans & Murshudov, 2013), with the exception of Wilson B-factor, ISa and CC_{1/2}, which were obtained from XSCALE (Kabsch, 2010). Absorbed X-ray doses were estimated using the program RADDOSE version 2 (Paithankar & Garman, 2010) and input parameters from Table S6. *Eiger 16M readout time between frames is 3 µsec (Casanas *et al.*, 2016), which corresponds to a 300 µsec total readout time for a complete dataset of 100 images, which is negligible relative to the 10 sec total collection time.

Temperature	Average Unit cell volume (\AA^3)	Standard deviation (\AA^3)
Proteinase K		
363	491.4	2.7
353	487.7	2.4
343	485.8	3.3
333	477.8	2.5
293	470.8	1.6
Thaumatococin		
313	531.1	N/A
293	525.8	N/A
Lysozyme		
323	229.4	N/A
293	225.1	N/A

Table S2. Data used for Figure 2A. The average volumes and standard deviations have been obtained from the data in Tables 1 and S1.

Temperature (K)	Unit cell volume (Å ³)								
	Imgs 1-10	Ave	S.D.	Imgs 91-100	Ave	S.D.	Imgs 361-460	Ave	S.D.
Proteinase K									
363	489.78	493.16	3.45	488.83	490.43	1.63	484.09	490.66	4.26
	489.95			490.06			489.98		
	494.98			493.14			493.15		
	497.91			489.69			495.44		
353	492.15	488.76	3.56	490.13	490.68	3.80	488.39	487.03	2.36
	491.04			489.63			489.34		
	488.89			496.72			487.24		
	482.94			486.22			483.16		
343	486.33	485.21	3.43	485.91	485.41	2.56	482.42	484.02	3.85
	481.91			483.46			480.83		
	490.33			489.39			490.61		
	482.25			482.90			482.23		
333	480.33	478.92	1.00	478.09	479.84	1.19	475.28	477.48	2.84
	479.30			480.19			479.39		
	477.67			481.40			481.07		
	478.37			479.67			474.17		
293	475.12	473.21	3.48	469.15	469.88	0.93	468.55	468.75	0.35
	476.19			471.18			469.25		
	468.32			469.29			468.46		
Thaumatococcus									
293	526.21			526.17			526.07		
313	531.13			530.98			536.67		
Lysozyme									
293	224.98			224.92			224.51		
323	229.98			228.76			228.33		

Table S3. Data used for Figure 2B. Unit cell volumes have been obtained using images 1-10 (beginning of the dataset), 91-100 (end of the dataset), and 361-370 (same orientation as for images 1-10 but after a complete 360° rotation). The additional comparison with images 361-370 was used as differences in unit cell parameters between images 1-10 and 91-100 could also originate from using different crystal orientations to estimate unit cell volumes. Unit cell constants have been obtained using a 2.0-Å resolution cut-off.

Temperature (K)	Average resolution (Å)	Standard deviation (Å)
Proteinase K		
363	1.63	0.05
353	1.52	0.15
343	1.38	0.01
333	1.38	0.10
293	1.24	0.22

Table S4. Data used for Figure 2C. The average resolutions and standard deviations have been obtained from the data in Tables 1 and S1.

Temperature (K)	Mosaicity (°)		
	Imgs 1-10	Average	S.D.
363	0.49	0.52	0.06
	0.48		
	0.5		
	0.62		
353	0.15	0.38	0.13
	0.48		
	0.47		
	0.41		
343	0.34	0.42	0.10
	0.51		
	0.52		
	0.3		
333	0.35	0.26	0.09
	0.23		
	0.12		
	0.34		
293	0.08	0.16	0.12
	0.07		
	0.33		

Table S5. Data used for Figure 2D. The average mosaicity and standard deviations have been obtained from images 1-10 of the diffraction data in Tables 1 and S1.

Temperature (K)	Proteinase K					Lysozyme		Thaumatococcus	
	293	333	343	353	363	293	323	293	313
Space group	P4 ₃ 2 ₁ 2	P4 ₃ 2 ₁ 2	P4 ₃ 2 ₁ 2	P4 ₃ 2 ₁ 2	P4 ₃ 2 ₁ 2	P4 ₃ 2 ₁ 2	P4 ₃ 2 ₁ 2	P4 ₁ 2 ₁ 2	P4 ₁ 2 ₁ 2
Unit cell (Å and °)	67.91 67.91 102.46 90.00 90.00 90.00	68.07 68.07 103.28 90.00 90.00 90.00	68.40 68.40 104.11 90.00 90.00 90.00	68.36 68.36 104.04 90.00 90.00 90.00	68.46 68.46 104.97 90.00 90.00 90.00	77.82 77.82 37.17 90.00 90.00 90.00	78.46 78.46 37.27 90.00 90.00 90.00	58.95 58.95 151.30 90.00 90.00 90.00	59.19 59.19 151.59 90.00 90.00 90.00
Wavelength (Å)	0.95369	1.03316	1.03316	1.03316	1.12709	1.03316	1.03316	1.03316	1.03316
Crystal size (µm ³)	300x300x250	300x250x200	300x250x200	350x300x200	300x300x250	300x250x200	350x350x250	350x300x100	300x300x250
Solvent content (fraction)	0.385	0.385	0.385	0.385	0.385	0.383	0.383	0.576	0.576
HA Ion, Concentration (mM)	Ca ²⁺ , 50	Ca ²⁺ , 50	Ca ²⁺ , 50	Ca ²⁺ , 50	Ca ²⁺ , 50	SO ₄ ²⁻ , 300	SO ₄ ²⁻ , 300	N/A	N/A
Beam size (µm)	270x80	270x80	270x80	270x80	270x80	270x80	270x80	270x80	270x80
FWHM (µm ²)	270x80	270x80	270x80	270x80	270x80	300x80	200x80	300x80	270x80
Flux (ph/s)	3.21e10 ¹⁰	3.14e10 ¹⁰	1.51e10 ¹¹	1.56e10 ¹¹	1.65e10 ¹¹	1.55e10 ¹¹	1.22e10 ¹¹	1.57e10 ¹¹	1.74e10 ¹¹
Collection time (s)	20	10	10	10	10	5	5	5	5
MaxDose (kGy)	14	8.3	40	42	52	23	27	26	28
AD _{G95} Dose (kGy)	8.3	4.8	23	25	30	15	13	18	16
DWD Dose (kGy)	4.1	2.4	11	12	15	7.6	6.7	8.4	7.9

Table S6. Crystal and beamline parameters used in RADDPOSE-3D absorbed dose calculations. Abbreviations used: Full width at half maximum (FWHM) for the beam; the worst case scenario dose – MaxDose (comparable to RADDPOSE v2); the average Diffraction Weighted Dose (DWD) for the absorbed dose; AD_{G95} represent average dose caused by a Gaussian beam over the crystal where 95% of the energy is deposited. The flux value was measured using calibrated Hamamatsu 300 microns thick Si photodiode and absorbed doses were calculated using RADDPOSE-3D (Zeldin *et al.*, 2013; Bury *et al.*, 2018).